Bioencapsulation Research Group

Bioencapsulation Innovations

April 2014

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22th International Conference on Bioencapsulation

September 17-19, 2014
Bratislava, Slovakia

http://bioencapsulation.net/2014_Bratislava/

EDITORIAL

Probiotics, or friendly bacteria, are good for you.

This simple message is promoted by health professionals, advocated by the media, supported by scientific research and valued by the industry. Most consumers would be familiar with their potential health benefits and may even be aware of the terms probiotics, prebiotics and synbiotics.

The Russian-born scientist Ilya Ilyich Mechnikov postulated over 100 years ago to possibly "modify the flora in our bodies and to replace the harmful microbes by useful microbes" as there was a clear "dependence of the intestinal microbes on the food" we eat. Since then, the area of bacteria and intestinal health has developed into a mature and exciting science, with plenty of ongoing research and development. The last 15 years have seen a sharp growth in the number of products on our shelves containing probiotic bacteria. According to the World Health Organisation probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host''. There is some disagreement about the adequate amounts, however the consensus is that probiotics must stay alive, and survive processing, storage and, in case of oral delivery, the harsh conditions of the gastric environment to reach the target in the intestine. However, probiotics are inherently unstable and the selection of probiotics has been mainly based on technological rather than bio-functional properties. One possible approach to overcome the high loss in viability is to protect, or to encapsulate the bacteria. There have been some success stories. However literature and patent offices are full of attempts to solve the problem. One of the main challenges in the area of probiotic protection is its multi-disciplinary nature. Microbiologists, chemists, technologists and food scientists from both academia and industry need to work together- and talk the same language.

The topic of the current newsletter is cell encapsulation and includes five short articles on probiotic encapsulation. Claude Champagne gives a comprehensive overview of the area covering encapsulation methods and technologies for food and supplements. Arthur Bartkowiak presents an example of encapsulation by spray-dying, Claire Gaiani introduces an interesting approach to understanding the neglected field of matrix/bacteria interaction, and Michael Cook gives a highly innovative microscopic technique to visualise acid penetration into gel beads. Finally Hani Al-Salami gives an overview of the vast area of cellular encapsulation, something that we may cover in a later newsletter.

As a guest editor of this newsletter, I sincerely hope that you- new, existing and potential members of the Bioencapsulation Research Group- find the current newsletter informative, possibly even inspiring. I also hope that this BRG Newsletter will help you meet potential partners, both academic and industrial, in the field of encapsulation.

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CALENDAR

PROGRAM 2014

May

24th Interdisciplinary Research Conference on Injectable Osteoarticular Biomaterials and Bone Augmentation Procedures
May 5-7, 2014 - Nantes, France
http://griboi2014.univ-nantes.fr/

Controlled & Modified Drug Release
May 7-8, 2014 - Philadelphia, PA, USA
http://curtiscoulter.com/events/controlled-modified-drug-release.html

Colloque MIS sur les Molécules et Ingrédients Santé
May 21-22, 2014 - Quimper, France
http://www.cbb-developpement.com/index.php?option=com_content&task=view&id=431&Itemid=650

8th International Conference on Polymer and Fiber Biotechnology
May 25-27, 2014 - Braga, Portugal
http://www.iptb.org

June

Functional Filmcoating
June 3-5, 2014 - Binzen, Germany
http://ttc-binzen.de/cm/index.php?id=681

8th World Congress on Polyphenols Application
June 5-6 , 2014 - Lisbon, Portugal
http://www.polyphenols-site.com

COSM'ING
June 26-28, 2014 - Saint Malo, France
http://www.cbb-developpement.com

July

Fluid Bed: Maintenance & Troubleshooting
July 1-7, 2014 - Binzen, Germany
http://ttc-binzen.de/cm/index.php?id=692

2nd Poorly Soluble Drugs Workshop
July 2nd , 2014 - Lille , France
http://www.apgi.org/2014_WS/

41st Annual Meeting & Exposition of the Controlled Release Society
July 13-16 , 2014 -Chicago, USA
http://www.controlledreleasesociety.org/MEETINGS/ANNUAL/

DynaCaps2014
July 15-18 , 2014 - Compiègne , France
http://www.utc.fr/dynacaps2014/

August

Translational Nanomedicine
August 27-29, 2014 - Angers, France
http://www.transnanomedicine.com

September

Success in Animal Cell Encapsulation
September 4-5, 2014 - Zurich, Switzerland
http://www.buchi.com/training

3rd International Meeting on Pharmaceutical Sciences
September 18-19, 2014 - Cordoba , Argentina
http://http://ricifa.com.ar

CALENDAR

	Bioencapsulation Research Group **22th International Conference on Bioencapsulation** September 17-19, 2014 - Bratislava, Slovakia http://bioencapsulation.net/2014_Bratislava/
	TTC TECHNOLOGY TRAINING CENTER **Continuous Granulation** September 23-25, 2014 - Binzen, Germany http://ttc-binzen.de/cm/index.php?id=714
Spetember	BENEFIQ 2014 **Benefiq2014** September 23-25, 2014 - Quebec, Canada http://www.benefiq.ca/en/
October	SFNano **SFNano Workshop 2014 : Relevant tools and models for translation of advanced drug delivery** October 2-4, 2014 - Porto, Portugal http://www.sfnano.fr/?page_id=3224&lang=fr
	TTC TECHNOLOGY TRAINING CENTER **Pan Coating** October 7-9,, 2014 - Binzen, Germany http://ttc-binzen.de/cm/index.php?id=719&L=0
November	Bioencapsulation Research Group **2nd South American Workshop on Microencapsulation** November26-28, 2014 - Joa Pessao, Brazil Web site available soon see http://bioencapsulation.net/
	TTC TECHNOLOGY TRAINING CENTER **Pellets and Micropellets for oral multi-dosage forms** October 25-27, 2014 - Binzen, Germany http://ttc-binzen.de/cm/index.php?id=769&L=

Bioencapsulation Research Group

22th International Conference on Bioencapsulation

&

21st Bratislava International Conference on Macromolecules

September 17-19, 2014 - Bratislava, Slovakia

http://bioencapsulation.net/2014_Bratislava/

Deadlines
For oral contributions and grant requests
June 15, 2014
For poster contribution
June 30, 2014

Bioencapsulation Research Group

2nd South American Workshop on Microencapsulation

November26-28, 2014 - Joa Pessao, Brazil

Web site available soon
see http://bioencapsulation.net/

ARTICLE

Encapsulated probiotic bacteria: one protection system is probably not enough

Claude P. Champagne – Food R & D Centre, Agriculture and Agri-food Canada. St. Hyacinthe, QC, Canada

Scope

Traditional microencapsulation (ME) methods, such as alginate beads, almost always improve the stability of probiotic bacteria during food processing, storage or in the gastro-intestinal (GI) tract. However, viability loses are often still too high for commercial application, even with ME. Therefore the efficacy of ME techniques needs to be improved. Recent trends point to combining them, and up to three protective systems have been applied. This manuscript will describe a few of the combinations possible.

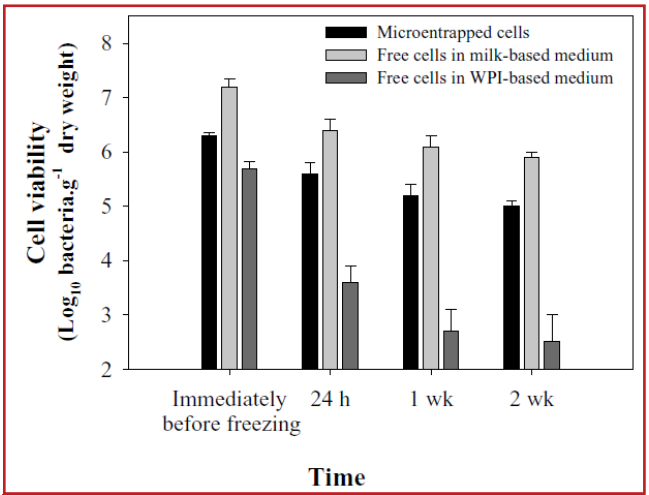

Figure 1: Changes in viable counts of free and microentrapped *Lactobacillus rhamnosus* cells in concentrated cranberry juice over 2-wk storage at –40°C (Reid et al. 2007).

Some Foods offer great challenges

As living entities, probiotic bacteria are sensitive to conditions of their environment, and show losses in viability in "stressful" conditions, such as heating, freezing, storage or exposure to acid. As an example, various dried cultures of *Lactobacillus rhamnosus* were added at 4.7 x 10^7 CFU/g (Log_{10} = 7.6) in a cold cranberry juice concentrate, and after only 15 minutes the viable counts had dropped by 0.5 to 1.7 Log CFU (Figure 1; data of "Immediately before freezing"). Further losses in viability occurred following freezing of the concentrate and during storage (Figure 1). In these assays, two parameters were tested: suspension medium of free cells and ME. A first observation is that milk was much more protective than a whey protein isolate (WPI) in protecting the cells. Secondly, ME improved the stability of the culture.

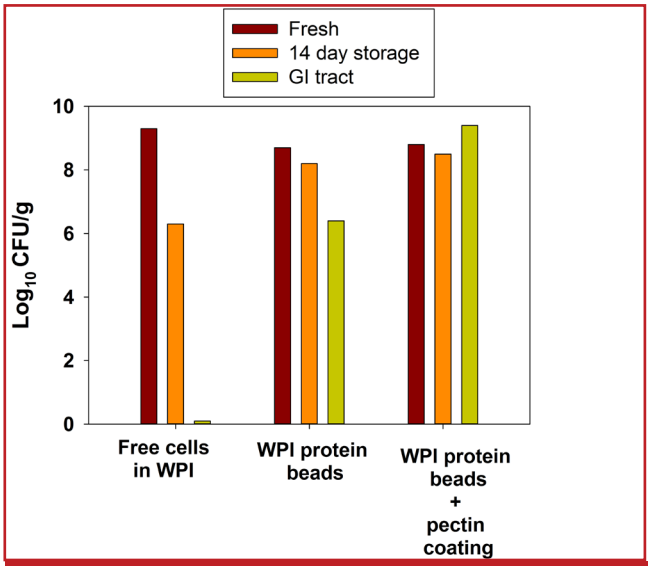

Figure 2: Effect of microentrapment in a whey protein (WPI) matrix and bead coating on viable counts of *L. rhamnosus* GG during storage and in a gastrointestinal environment. Figure redrawn from data of Doherty et al. (2012).

This data is quite typical in the field of probiotics. The trend today is to have a billion cells per portion. In the case of the cranberry juice concentrate, a portion or 100 mL would have resulted in 4.7 x 10^9 CFU, and would have answered this target. But the cell count became too low as soon as the product was frozen. Even though ME in WPI increased the survival rate by a factor of 100 (Figure 1), it is still not enough to allow marketing of the product. After 14 days only 1 out of every 1 million cells added had survived the process. The observation that the cells suspension medium itself has a major impact, even more than ME, has been demonstrated by Saarela et al (2006). These data evidence the challenge of keeping probiotics viable in some food matrices, and the limits of some protection strategies. In many cases, a "single" protective system is just not enough.

Coating a microparticle

Alginate and whey proteins are polymers which have charged secondary radicals. Therefore it is possible to add, at the surface of microbeads, coats of polymers having an opposite electrostatic charge. The classic example is an alginate bead with a chitosan coating. But newer systems are developed, and proteins are gaining in favour. In order to better illustrate the benefits of coating and multiple protection approaches, it was logical to use a food matrix similar

Table 1. A few examples of multiple coatings or multiple protection systems for probiotics based on microencapsulation.

Components - Levels			Reference
Alginate - Microentrapment	--> Coat 1: Poly-L-Lysine	--> Coat: 2 Alginate	Martoni et al (2011)
Whey protein – Microentrapment	--> Coat 1: Pectin	--> Coat 2: Whey protein	Doherty et al (2012)
Alginate – Microentrapment	--> Coat 1: Poly-L-Lysine	--> Coat 2: Oil	Ding and Shah (2009)
Freeze-dried culture	--> Coat 1: Oil (spray-coating)	--> Coat 2: Chocolate or lipid	Champagne et al (2011)

ARTICLE

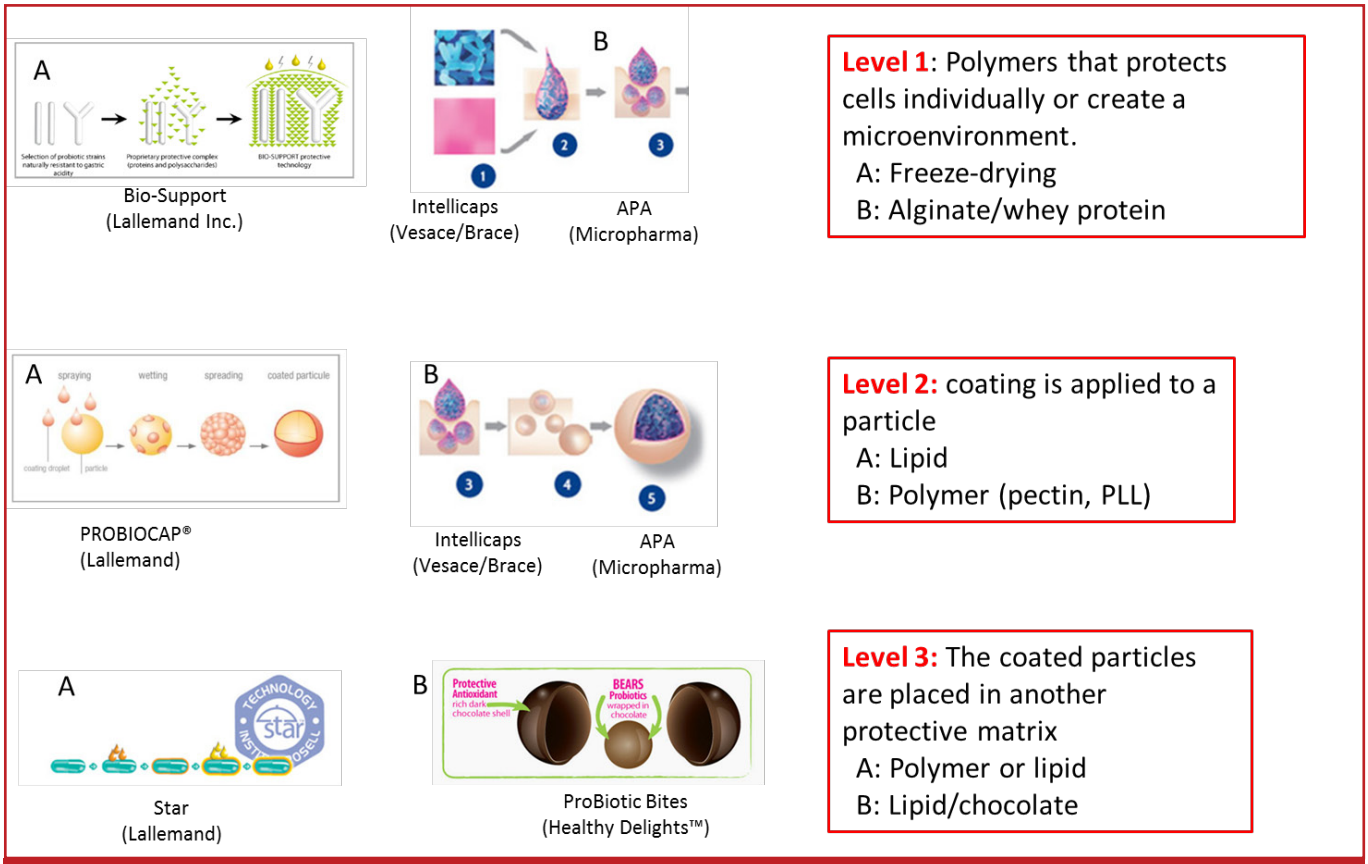

Figure 3: Examples of products with multiple protection elements that are commercially available.
From: (www.lallemand-health-solutions.com ; www.intelicaps.net/en/intelicap ; www.probioticbites.com; www.micropharma.net)

as that described in Figure 1, as well as the same bacterial species (*Lactobacillus rhamnosus*). As was the case in Figure 1, free cells in a cranberry juice die very rapidly, and micro-entrapment helps to reduce the mortality rates (Figure 2). But it is when the protein-based particle is coated with pectin that the system becomes highly efficient (Figure 2). And additional coatings are possible in the same fashion (Doherty et al., 2012). We are now seeing not only multiple protection approaches but some result from different technologies (Figure 3; Table 1).

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada
Food Research and Development Centre | Centre de recherche et de développement sur les aliments

The future?

If the highly successful results of Doherty et al (2011) can extend to these other systems, applications in foods should be considerably favoured. Furthermore, it is to be hoped that these systems could reduce the effect of the food matrix on probiotic functionality in the GI tract, and open the way for the transfer of health allegations between food matrices.

References

Ding WK, Shah NP, 2009. An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. Journal Food Sci. 74, M53-M61.

Doherty SB, Auty MA, Stanton C, Ross RP, Fitzgerald GF, Brodkorb A. 2012. Application of whey protein micro-bead coatings for enhanced strength and probiotic protection during fruit juice storage and gastric incubation. Journal Microencapsul. 29, 713-728.

Martoni, C., Jones, M.L., Prakash, S., 2011. Epsilon Poly-L-Lysine capsules. Patent WO/2011/075848 (PCT/CA2010/002057

Reid A, Champagne C, Gardner N, Fustier P, Vuillemard JC. 2007. Survival in food systems of L. rhamnosus R011 microentrapped in whey protein gel particles. J Food Sci, 72: M31-M37.

Saarela M, Virkajarvi I, Nohynek L, Vaari A, Matto J, 2006. Fibres as carriers for Lactobacillus rhamnosus during freeze-drying and storage in apple juice and chocolate-coated breakfast cereals. Int. J. Food Microbiol. 112, 171-178.

Claude P. Champagne

Claude P. Champagne is a research scientist at Agriculture and Agri-food Canada. He specialises in the technological aspects of production of lactic and probiotic cultures, as well as their use in food fermentations.

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ARTICLE

A Better understanding of probiotic / milk proteins interactions: a way to enhance bacterial encapsulation efficiency

C. Gaiani, J. Burgain, J. Scher, Univ. de Lorraine, LIBio, Vandoeuvre les Nancy

Introduction

Probiotic dairy powders

The main techniques used to obtain powders (spray-drying, freeze-drying...) have not changed, or not much, but the product has considerably. In the 1950's, dairy powder was only a form of storage for animal foodstuff ("first generation" powders) and only the milk surpluses were dried. Twenty years later appeared milk extraction and separation methods using membrane technology, which produced "second generation" powders (Jourdain, 2012). Nowadays, more than twenty constituents are recovered from milk. These are the "third generation" powders, referred to as assembly powders. Among them, infant formula powders are a booming market, with a two digit growth throughout the world. There is presently a significant stake in the development of a "fourth generation" powders for nutritional or medical purposes. The purification of some proteins (lactoperoxidase, lactoferrin...), protein's hydrolysis for the production of biopeptides, or the introduction of probiotics can be mentioned as examples of "fourth generation" powders.

LIBio
Laboratoire d'Ingénierie des Biomolécules

This work is precisely focused on dairy probiotic powders presenting high added value of strategic importance to the competitiveness of European industrial sectors (functional food and beverage suppliers and manufacturers as well as dietary supplements, specialty nutrients and animal feed industries). On a global scale, the probiotics market is mainly driven by the rising popularity of probiotic functional foods and beverages among consumers. Dynamics are supported by links between food and health. Age, stress, poor diet are some of the reasons responsible for digestive ailments, bloating, reduced resistance to infections, and consumption of probiotic helps to alleviate these widespread conditions. Consequently, global sales of probiotic foods amounted to $21.6 billion in 2010, $24.23 billion in 2011 and are expected to reach $31.1 billion by 2015 (Pedretti, 2013).

European probiotic foods: a unique market

Among the long list of health benefits that have been attributed to probiotics; the scientific background supporting those benefits is still controversial in Europe. Probiotic bacteria are defined as "live microorganisms which when administrated in adequate amounts can provide a health benefit on the host" (FAO, 2001). However, to exert these beneficial effects, probiotic bacteria must maintain their viability first during food processing and storage and then during their passage through the upper gastro-intestinal tract in order to arrive in the intestine in a viable state. Due to new European regulations, the probiotics market will slow down in Europe (€5.13bn last year to about €5bn in 2017) but will keep a strong dynamic in Asia-Pacific, North America and rest of the World. Indeed, the endorsement of this regulation has seriously affected the market and the industrialists' communication. Under the terms of this regulation, the viability of cultures in the functional food must be assessed.

Milk proteins/bacterial interaction must be assessed

Up to now, many researchers have focused their activity on the microencapsulation of probiotic bacteria into dairy matrices aiming at protecting the cells for a controlled release in the gut. However, these studies only recorded parameters like encapsulation yield or survival rate, with differences being observed when changing the dairy matrix or the encapsulation technique. Dealing with these observations, the way the bacteria interact with the dairy matrix was less investigated. This aspect is vital to understand how the delivery performance could be improved and to transpose the result to other bacterial strains. In this study, the encapsulated strain is *Lactobacillus rhamnosus* GG (LGG). LGG is one of the best documented probiotic strain with proven health benefits that include the prevention and relieve of acute diarrhea, the prevention of antibiotic-associated diarrhea and the prevention of atopic disease. Consequently, this work deals with the elucidation of some interactions occurring between milk proteins / bacteria and bacterial location within the particle.

Results and discussion

Why to use of Atomic Force Microscopy (AFM)?

Many bacteria present appendages on their surface which allow microorganisms to interact with different substrates. Therefore, it is necessary to develop methods capable of probing the surface properties of microbial cells at a molecular scale in order to refine the traditional view of the process of microbial adhesion. AFM can localize and manipulate individual molecules, this technique is known as single-molecule force spectroscopy (SMFS). By labeling the tip with specific antibodies or ligands, single molecule can be detected in complex environments such as living cells (Burgain, 2013). Bacteria were electrostatically immobilized on the AFM tip for force spectroscopy measurements and milk proteins were deposited on mica surfaces (Fig. 1).

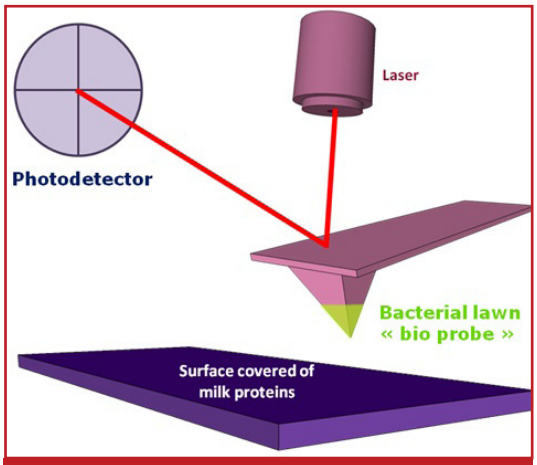

Figure 1: Principle of Force measurement with AFM

Interaction between LGG and caseins

In bovine milk, the four caseins (αs1- αs2- β- κ-caseins) are assembled together to form a structure called micelle. These proteins do not have a well-defined tertiary structure. Interactions between casein micelles

and biomolecules are of interest because the interior of the casein micelles is not accessible for large molecules whereas, it is porous enough to provide access to the inner part of the structure for small molecules (eg. enzymes).

Non-specific events were reported for interactions between LGG and caseins. This was observed by the single adhesive event presenting a length lower than 100 nm (Figure 2).

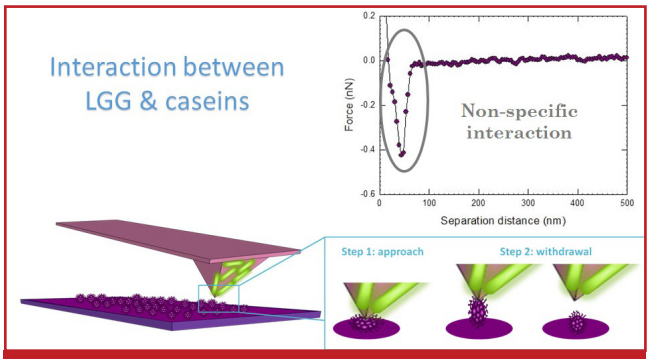

Figure 2: Interactions between LGG and caseins (Burgain, 2013)

Interaction between LGG and whey proteins

Whey proteins have a defined tertiary structure accessible to other biomolecules. This explains why specific interactions can be established between molecules that decorate bacterial surfaces and whey proteins. It was established that interactions between LGG with whey proteins are specific in nature. In fact, multiple adhesive events were observed on the retraction curve when determining forces by AFM (Figure 3).

Conclusion and outlooks

The adhesive nature of bacteria is mainly due to various cell surface features consisting of proteins such as pili, and polysaccharides.

In order to go further and to identify specifically which biomolecules at the surface of LGG interact with the whey proteins, some mutants derived from the LGG wild-type strain were selected (collaboration with Lebeer et al., 2009; 2012):

- spaCBA (a depleted pili strain that lost its capacity to bind human intestinal mucus),
- welE (an exopolysaccharide deficient mutant which could increase surface accessibility for adhesion proteins)
- dltD (a mutant presenting drastic modification in its lipoteichoic acid molecules, absence of D-alanyl esters).

By applying models to the AFM retraction curves and comparing retraction curves obtained for the wild-type strain to the ones obtained for mutants; the exact identification of biomolecules allowing the interactions between milk proteins and bacteria will be identified. Depending on the molecule identified (pili, exopolysaccharide...), it will be easier to extend our knowledge to other strains.

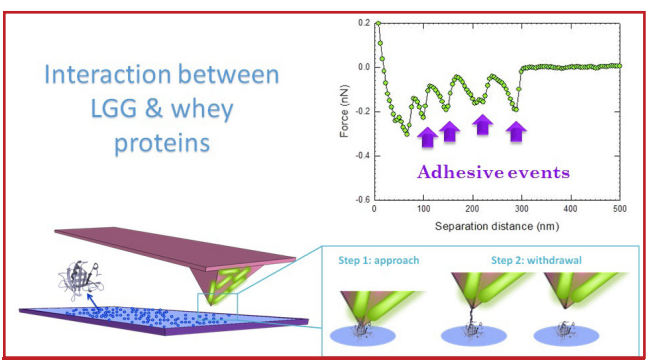

Figure 3: Interactions between LGG and whey proteins (Burgain, 2013)

References

J. Burgain, C. Gaiani, G. Francius, , A.M. Revol, C. Cailliez-Grimal, S. Lebeer, H.L.P. Tytgat, J. Vanderleyden & J. Scher (2013). In vitro interactions between probiotic bacteria and milk proteins probed by atomic force microscopy. Colloids and Surface B: Biointerfaces, 104, 153-152.

J. Burgain (2013). Microencapsulation de bactéries probiotiques dans des matrices laitières: Etude des mécanismes de formation par une approche multi-échelle. PhD, Université de Lorraine.

FAO/WHO (2001). Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. 2001 Report of a Joint FAO/WHO Expert Consultation. Available at: *http://www.fao.org/es/ESN/food/foodandfoo_probio_en.stm.*

F. Jourdain (2012). L'industrie laitière investit massivement dans les produits secs, in: L'agroalimentaire au quotidien.

S. Lebeer, I. Claes, H. Tytgat, T.L.A. Verhoeven, E. Marien, I. van Ossowski, J. Reunanen, A. Palva, W.M. de Vos, S.C.J. Keersmaecker & J. Vanderleyden (2012). Functional analysis of LGG pili in relation to adhesion and immunomodulatory interactions with intestinal epithelial cells Applied and Environmental Microbiology, 78, 185-193.

S. Lebeer, T.L.A. Verhoeven, G. Francius, G. Schoofs, I. Lambrichts, Y. Dufrêne, S.C.J. Keersmaecker (2009). Identification of a gene cluster for the biosynthesis of a long, galactose-rich exopolysaccharide in LGG and functional analysis of the priming glycosyltransferase Applied and Environmental Microbiology, 75, 3554-3563.

S. Pedretti (2013). Probiotic market: up or down? Nutrafoods, 12, 18-19.

Dr. Claire GAIANI

Claire Gaiani obtained her Dairy Food Science diploma of Engineer from the Polytechnique Institute of Lorraine. Her PhD was focused on the development of methodologies allowing the characterization of milk powder rehydration under an industrial contract with the French dairy Industry (CNIEL). Currently, she is Associate Professor at the University of Lorraine. Her researches deal with the vectorisation of bioactives in dairy matrices and the multiscale characterization of food powders in order to better understand the links between processes, structures and functionalities.

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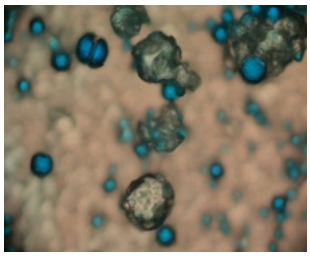
Forming pure spheres in space



The Microencapsulation Electrostatic Processing System-II (MEPS-II) was first trialled in space by NASA in 2002 and has made a return journey in a project to investigate novel cancer therapies. Carrying out droplet formation in space enabled the mixing together of immiscible liquids with quite different densities without the formation of layering that would occur under gravity. Subsequent film forming at a liquid/liquid or air/liquid interface can then occur resulting in the production of perfectly formed spherical droplets. Resulting microcapsules should provide a more reproducible and controlled delivery profile than equivalent materials currently produced under gravity. Additional development work is underway to ensure the process is scalable. The microencapsulation technology is now under license to NuVue Therapeutics, Inc who is embarking on clinical trials.
http://www.nuvuetherapeutics.com/

Further information: http://1.usa.gov/1gC6nnA.

Why not including information about your company here. This newsletter is your tool for communicating with 7000 persons

ATP-Mediated drug delivery to fight cancer

UNC NCSU BME BIOMEDICAL ENGINEERING

Adenosine-5'-triphosphate (ATP) is being used as the trigger to initiate drug delivery from doxorubicin loaded cross-linked hyaluronic acid gel-shell microcapsules delivered to tumours. DNA is complexed with doxorubicin within the nano-capsules cells which are selectively adsorbed onto the surface of the cancer cells. The nano-carrier is then absorbed by the cell where the presence of high levels of ATP triggers a conformational change in the DNA molecules releasing the doxorubicin. Although at an early stage, only in vitro studies have been carried out to date, researchers at North Carolina State University and the University of North Carolina at Chapel Hill saw a 3.6 fold improvement in drug activity when the ATP mediated trigger mechanism was utilised.

More information: http://bit.ly/1rE4l9Z

Targeted delivery of interleukin-2 (IL -2) for cervical cancer treatment is being achieved using nanoparticulates

Researchers at the Laboratory of Cellular Oncology of the Faculty of Higher Studies (FES) Zaragoza UNAM (National Autonomous University of Mexico) aim to provide a more effective therapy by coating cationic liposomes with IL-2 that will bind to the surface of the cancer cell. *In vitro* and *in vivo* studies have shown promising results.

More information and an abstract of the original research can be found here http://1.usa.gov/1o3l7Pk.

Migration of nanoparticles from packaging into foodstuffs investigated

Iranian researchers have investigated the migration patterns of clay nanoparticles incorporated into nano-composite polyethylene terephthalate (PET) bottles. Inductively coupled plasma spectroscopy revealed increased levels of silicone and aluminium in the acidic food model matrix and X-ray diffraction analysis, transmission electron microscopy (TEM) and atomic force microscopy (AFM) showed evidence of intercalation and exfoliation morphology in the PET/clay nanocomposites.

An abstract of the original paper can be found at http://bit.ly/1duQsa4.

Alco-PIP technology from Encapsula

A process to incorporate alcohol into "Polymer Impregnated Particles" (PIP) has been developed by UK company Encapsula Limited which is ready to scale up and offer products to the food and beverage industries. A free flowing powder of 30-35 micron diameter particles can be produced with a microcapsule loading of up to 50% w/w alcoholic solution claims the company. Proposed applications include packaging and labelling coatings with a sustained delivery of aroma triggered by touch.

More information at http://bit.ly/1paQ2ay and at the company's website at http://www.encapsula.co.uk/

Feeding the Planet, Energy for Life

Expo 2015 will be an extraordinary universal event displaying tradition, creativity and innovation in the business of food. It will bring together many themes that have already been handled by this event in the past, and set them out anew in light of new global possibilities whose common core is the idea that everyone on the planet should have access to food that is healthy, safe and sufficient.

Workshop and debate themes foreseen by the Expo organization include many topics Agro Food Industry hi Tech journal is familiar with. Please go to www.teknoscienze.com to learn more on our publication. Our Journal closely follow academic and industry work in the world of food science, reporting on innovation in R&D, market trends and regulations. Our authors are distinguished opinion leaders coming both from Industry and University. **We are currently preparing the 2014 issues of the journal and are looking for technical papers on Flavours, encapsulation and dietary supplements**. Deadline for submission, May 5th. Authors' guidelines available on our website.

Authors are invited to present case studies in their field with an effort to share perspectives and solutions on the many themes which will be addressed during EXPO (for a complete list of topics please refer to EXPO website at *http://en.expo2015.org/expo-2015*).

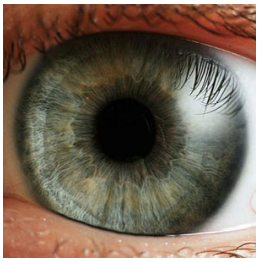
Further information: dr Gayle De Maria at gayle@teknoscienze.com

Southwest Research Institute (SwRI) receives project boost

The SwRI has been awarded $8.3 million by the U.S. Department of Health and Human Services Biomedical Advanced Research and Development Authority (BARDA) to continue the development of a nasal-delivery system. The aim is to use an intranasal delivery formulation of iso-amyl nitrate as a first line treatment for cyanide poisoning. The project received an initial $4.4 million in funding in 2011. The treatment would enable an untrained individual to quickly and safely administer amyl nitrate to a victim of poisoning instead the need to wait for the application of an intravenous dose by trained personnel.

Further information: http://bit.ly/1hXDleJ and on the SwRI website: http://www.swri.org/9what/releases/2014/BARDA-cyanide.htm

New nanotechnology treatment for AMD in development



Treatment of age-related macular degeneration (AMD) is typically by invasive intraocular injection that carries risks of infection and haemorrhage. Professor Francesca Cordeiro of the University College London (UCL) Institute of Ophthalmology and a team of UK scientists have developed a liposome based formulation of bevacizumab (Avastin™) that utilizes annexin A5 mediated endocytosis to facilitate drug delivery. This means that in future a range of therapies could be applied to the eye as eye-drops.

Further information on the UCL website at http://bit.ly/1m7FSWu. An abstract of the original paper can be found at http://bit.ly/1m7FHKU.

Response Scientific, Inc. launches their Microvail™ encapsulation technology into the nutritional supplements marketplace

Response Scientific aim to launch their new products in Canada and the US in 2014/15 (http://bit.ly/1l3ILI4). The life sciences and biotechnology company have a patented microfluidics based microencapsulation technology that they are also developing to provide oral delivery insulin dosage forms.

More information at http://bit.ly/1gpulff and on their website at http://www.responsescientific.com/.

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ARTICLE

Visualising pH within alginate gels using confocal microscopy

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Introduction

Probiotic bacteria have become popular in recent years due to their perceived ability to improve gut health. However, the acid sensitivity of many strains of probiotic reduces the amount of bacteria surviving passage through the stomach. As only live bacteria are believed to exert potent health benefits in vivo, this cell death reduces the efficacy of any oral probiotic administration, as well as yielding some sensitive strains of bacteria completely ineffective.

The microencapsulation of probiotic bacteria into alginate gels has been utilised as a method to protect the cells during exposure to low pH solutions, such as those found within the stomach (Cook et al, 2012). However, the mechanism by which alginate protects these sensitive cells is not fully understood. In a collaborative project between the Food and Pharmacy departments at the University of Reading, a method has been developed to visualise the pH within alginate gels during exposure to acidic solutions (Cook et al, 2013). This method involves the tagging of bacteria with two pH sensitive fluorophores, and subsequent visualisation of the cells by confocal microscopy. By independently exciting each fluorophore, the pH experienced by the bacteria can be determined ratiometrically. Using MATLAB®, this data can be transformed into coloured images, allowing the production of 'pH maps' showing the distribution of acid within the gels.

Fluorescent bacteria as a pH probe – the technique

In our study (Cook et al, 2013), we used simple procedures to tag a probiotic strain, *Bifidobacterium breve*, with two pH sensitive fluorophores (Figure 1). These fluorophores, pHrodo and fluorescein isothiocyanate (FITC), are both pH responsive, increasing their fluorescence as pH is decreased or increased, respectively. Suspending these fluorescent bacteria in solutions with pH 2-7 and independently exciting each fluorophore using a confocal laser-scanning microscope allowed the production of a calibration curve based on the fluorescence intensity of pHrodo divided by that of FITC. These bacteria were then encapsulated into alginate gels using an extrusion and ionic gelation technique. As alginate gels are translucent, a cross-section of the material may then be observed by confocal microscopy. If two sequential cross-sections are taken of the material at the excitation wavelengths of each dye, two images may be produced showing the fluorescence intensity of each fluorophore throughout the material. Dividing the pixel values of the image taken at the pHrodo wavelength by the image at the FITC wavelength can simply be done using on-board 'Boolean logic' transformations. The end result is an image showing the ratio of pHrodo fluorescence divided by FITC fluorescence. By importing the image into MATLAB®, this data can be transformed into a matrix of numbers reflecting the fluorescence intensity of each pixel in the image. From this point, fluorescence ratios can be transformed into pH values using the previously established calibration curve and the data binned in such a way that pixels lying within certain pH ranges are designated a specific colour. The end result is a 'pH map', which colour-codes the image allowing easy visualisation of the pH distribution within the material.

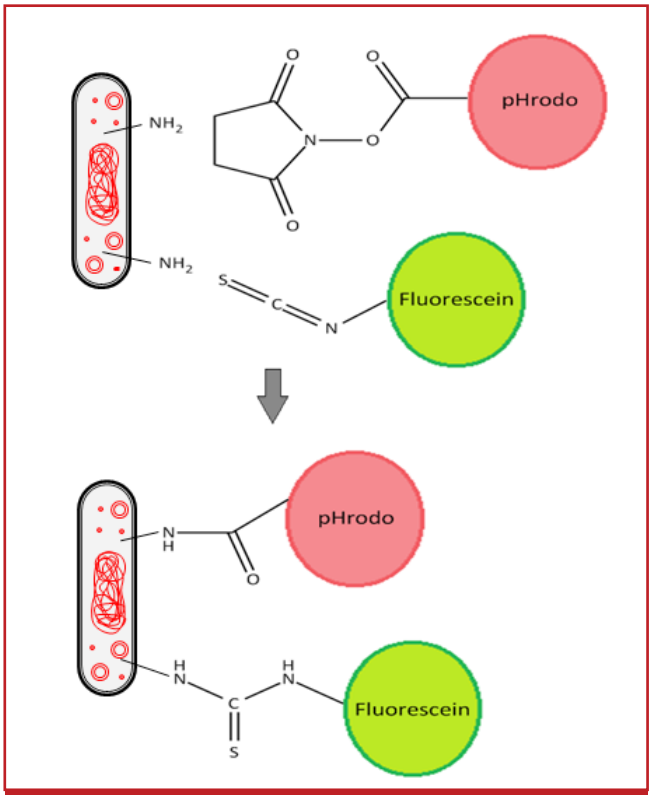

Figure 1: Labelling of bacteria with pHrodo and FITC. Adapted with permission from Biomacromolecules 2013, 14 (2), pp 387–393 . Copyright (2013) American Chemical Society

Visualising the ingress of acid using microscopy

Once a method for producing pH maps had been established, it could be utilised to show the ingress of acid into the alginate gels (Figure 2). Encapsulated fluorescent bacteria were mixed with a simulated gastric solution (pH 2), and images taken by confocal microscopy at regular intervals over one hour. This was performed on alginate gels, and alginate gels coated with the cationic polysaccharide

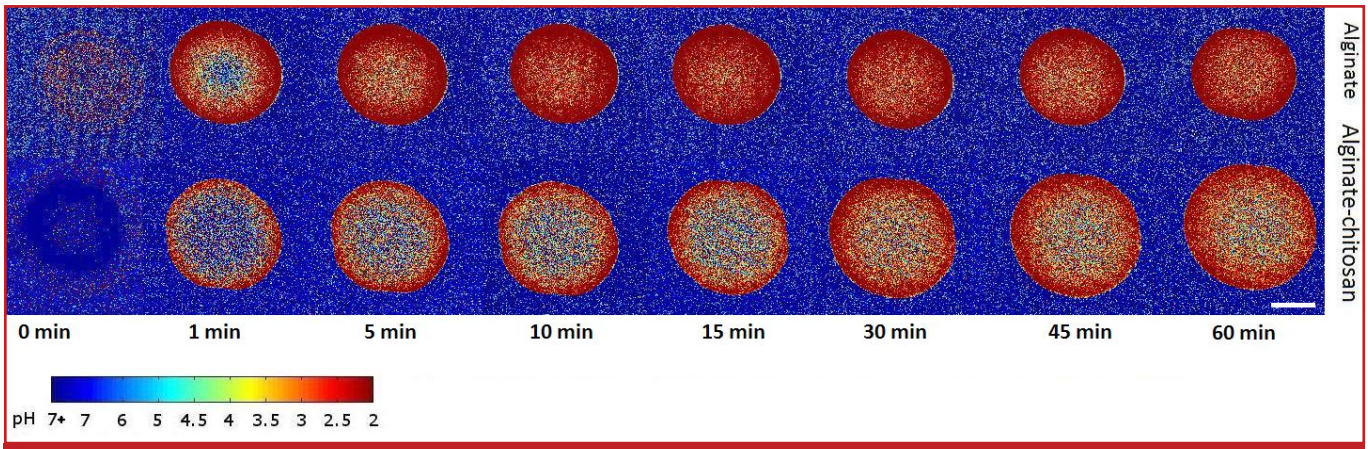

Figure 2: Visualising the penetration of acid into alginate (top) and alginate-chitosan (bottom) gels during exposure to a pH 2.0 solution. Reprinted with permission from Biomacromolecules 2013, 14 (2), pp 387–393 . Copyright (2013) American Chemical Society.

chitosan, which has been shown to improve cell survival in acid. The resulting images clearly show a layer of red, associated with pHs approaching 2, appearing first at the periphery, before further penetrating into the material. This is most easily seen by looking at the spot of lighter colour in the centre of the image. The rapid onset of acid penetration in the early stages of acid exposure, seen by the dark red ring at the periphery, followed by the slower penetration over the rest of the hour implies a change in the material properties of the gel. It is known that alginate forms an acid gel at pHs below 4 (Draget et al, 1994), so the slowing of acid penetration was hypothesised to be due to this effect. Briefly, the reduction in pH allows for protonation of carboxylate groups on alginate, which results in the aggregation of polymer chains and reduced porosity. If alginate gels were not pH-responsive then a more homogeneous pH change may be expected.

Comparing the alginate gels with chitosan-coated alginate gels allows the identification of a region of yellow/blue inside the chitosan-coated alginate gels at times of up to 1 h. This region is at a pH greater than 3.5, so will be considerably less harmful than the pH 2 solution that the gels sits in. This region of higher pH is also seen in the alginate capsules, just to a smaller extent. Due to the presence of a region of higher pH, a greater number of cells will survive inside the capsules than would be found with unencapsulated cells. This increase in survival was verified by viable counting (Cook et al, 2013) The decrease in the ingress of acid into chitosan-coated alginate gels is likely to be due to either reduced porosity, or an increased buffering capacity of the material in the regions containing chitosan.

Conclusions and outlook

A method has been developed which allows the production of pH maps using confocal microscopy. This has been applied to alginate gels in order to validate the method and to increase understanding of the processes occurring within the material during exposure to acid. It appears that acid penetrates into the alginate gels in a time-dependent fashion, reducing the exposure time of bacteria to the harmful low pH. This slowing of acid penetration was more significant for chitosan-coated alginate gels, possibly due to a reduction in porosity, or increase in buffering capacity.

This technique could be useful in the evaluation of many enteric dosage forms, as long as they are of sufficient translucency to be visualised by confocal microscopy. Thus, hydrogels and closely-related materials are ideal for this technique. The microbe used may be altered as the labelling technique is very general. Additional pH probes will be investigated by the group in time.

References

Cook, M.T., Tzortzis, G., Charalampopoulos, D., Khutoryanskiy, V.V.; Microencapsulation of Probiotics for Gastrointestinal Delivery; Journal of Controlled Release, 2012, 162 (1), 56-67.

Cook, M.T., Saratoon, T., Tzortzis, G., Edwards, A., Charalampopoulos, D., Khutoryanskiy, V.V.; CLSM Method for the Dynamic Observation of pH Change within Polymer Matrices for Oral Delivery; Biomacromolecules, 2013, 14 (2), 387-393.

Draget, K.I., Braek, G.S., Smidsrod, O.; Alginic Acid Gels - the Effect of Alginate Chemical-Composition and Molecular-Weight.; Carbohydrate Polymers, 1994, 25, 31-38.

Michael T. Cook

Michael Cook is a postdoctoral research assistant at the University of Reading. He was awarded his doctorate entitled "Microencapsulation of a Synbiotic Product" in July 2013 from the University of Reading. His research interests are focused on the application of natural and synthetic polymeric materials to pharmaceutics. He is currently most active in the area of probiotic microencapsulation, hydrogel synthesis and characterization, and mucoadhesion.

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ARTICLE

Encapsulation of probiotic microorganisms by spray-drying technique

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INTRODUCTION

Probiotic microorganisms, defined as mono or mixed culture of live bacteria or yeasts, when ingested in certain numbers, exert health benefits beyond inherent basic nutrition. The reported therapeutic or nutritional values of probiotics include suppression of growth of pathogenic microflora, increased bioavailability of certain vitamins and minerals, reduction in the risk of cancer, improvement of lactose digestion and immune system activity or decrease in cholesterol levels of the host (Chen and Chen, 2007). The most important probiotics known to have beneficial effects on the human gastrointestinal tract are Lactic Acid Bacteria (LAB), however the effects of probiotics are strain specific, which in turn affects the necessary dose levels (Kekkonen et al., 2007). The generally accepted number of probiotic microorganisms ingested daily is between 10^6 and 10^8 CFU/g.

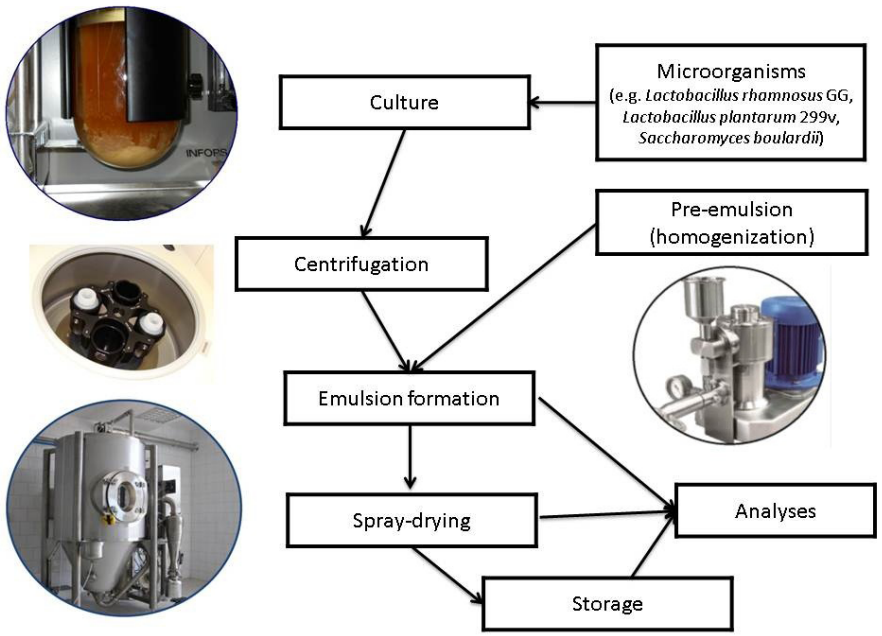

Fig. 1. Schematics for the process of spray-dried probiotic preparation

Nowadays the consumers not only expect their food to fulfil their immediate nutritional needs but also to provide additional specific health benefits. This is one of the reason for rising popularity in probiotics health-based products. One challenge is to develop probiotic preparation containing high number of live cells of microorganisms which maintain their viability for a required time. Another challenge is to develop a preparation for food application in a form facilitating its distribution, storage and dosage. For this reason the process of spray-drying and culture conditions were optimized to increase the efficiency of the encapsulation process and the viability of probiotic bacteria. The standard procedures involve the production of preparations containing probiotic bacteria only or probiotics together with prebiotic substances. A different approach was undertaken in our research group where the probiotics were encapsulated together with polyunsaturated omega-3 fatty acids to enhance the beneficial properties of the resulting product.

Microencapsulation by spray-drying

Spray-drying is commonly used method of encapsulation in the food industry (Picot and Lacroix, 2004). It involves atomization of an emulsion of probiotic microorganisms and carrier material into a drying chamber, when the water rapidly evaporates. The resulting product has a powder form, and the whole process is rapid and of relatively low cost.

Our approach was to optimize the conditions for culturing of microorganism and spray-drying to enhance probiotics viability during the whole process, as well as to determine the best conditions for further storage of the preparation. An important aim of our research was also to develop a stable emulsion containing proper carrier substances, probiotics and omega-3 fatty acids that will guarantee the highest quality and sensory properties of final dried preparation. Our strategy involved both the laboratory and ¼ technical scale. The probiotic microorganisms tested included several Lactobacillus sp. strains and probiotic *Saccharomyces boulardii*. The overall workflow is presented in Fig. 1. The strains were cultured in a bioreactor, the microbial biomass was retrieved by centrifugation and mixed with pre-emulsion containing carrier suspension, omega-3 fatty acids and antioxidants to form final emulsion, which was subjected to spray-drying process. The obtained dry products was stored and analysed during 6 months of its storage in different conditions, when microbial quality and sensory analyses were performed.

Factors affecting probiotics survival

Culturing conditions of microorganisms seem to be an important factor influencing their further survival rate during spray-drying process. Regardless of other factors, in case of tested microbial strains the highest survival after spray-drying was observed for microorganisms harvested during the stationary phase of their growth, whilst the lowest was noted for those in logarithmic phase of growth. The optimal time to harvest microbial cells was strain – dependent, and for example in case of *Lactobacillus rhamnosus* GG strain it was between 20 and 22h of culture, whilst for *Lactobacillus plantarum* 299 – between 18 and 21h of culture (34°C).

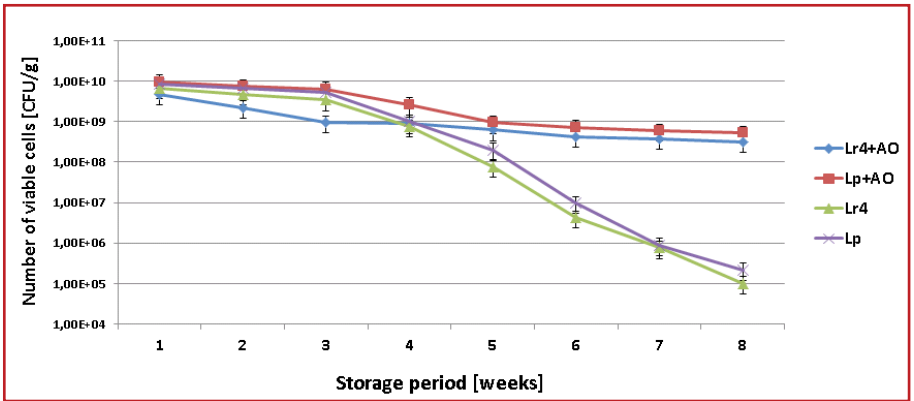

Fig. 2. Effect of antioxidant (AO) addition on the survival of probiotic *Lactobacillus rhamnosus* (Lr4) and *Lactobacillus plantarum* (Lp) in stored spray-dried preparations.

Composition of emulsion subjected to spray-drying process greatly influences the survival of probiotic bacteria. Standard carrier materials used in spray-drying are natural and modified polymers, such as polysaccharides and proteins. We obtained optimal results when using modified soluble starch at the amount of 9 – 11% dry weight combined with 2 maltodextrins (with dextrose equivalent DE=10 and 18) at the total amount of 3 – 4% dry weight. The emulsion also contained polyunsaturated omega-3 fatty acids and natural antioxidants (herbal oils and extracts) to prevent PUFA from oxidation. Even the addition of 0,2% antioxidant significantly positively affected the survival of probiotic microorganisms in spray-dried preparation, and the effect was visible after 5 weeks of storage (Fig. 2).

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Bacterial survival and their viability were highly dependent on the conditions of spray-drying process, especially the cyclone outlet temperature. As seen in Fig. 3 the outlet temperature exceeding 85°C had a negative influence on the survival of probiotic microorganisms in the obtained powder, however this effect was more pronounced in yeasts than bacteria. Overall survival rate after spray drying process was strain – dependant, with the highest survival observed for *L.rhamnosus* GG. The number of live cells in obtained spray-dried preparations exceeded 10^9 CFU/g (Fig. 4). Probiotic microorganisms encapsulated by spray-drying were also observed by SEM to determine the shape and size of microcapsules (fig. 5).

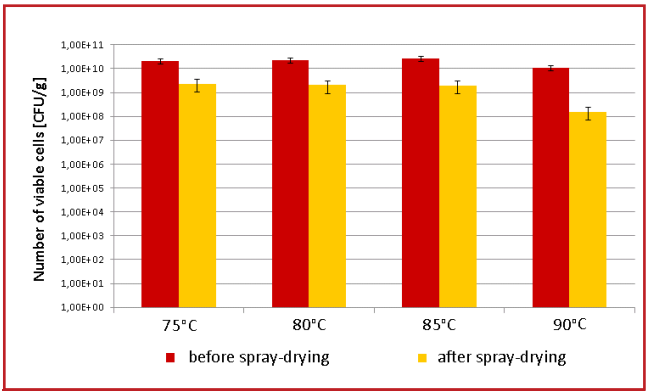

Fig. 3. Effect of outlet temperature on the survival of probiotic *Lactobacillus rhamnosus* GG.

The obtained probiotic preparations were stored at 4, 8°C and 24°C to determine the viability of spray-dried probiotics in specific conditions. The samples were stored in normal and modified atmosphere, the latter being 75% N_2 with 25% CO_2. The use of MAP and low temperature storage positively affected the survival rate of probiotic microorganisms in obtained preparations, and the use of both MAP and temperature 8°C or below exceeded the shelf life of the preparation twofold, without loosing its quality.

Summary and Conclusion

Food is considered an ideal vehicle for delivering the probiotics to the human gastrointestinal tract. The viability of probiotic cells is of the highest importance because to have beneficial effect on health they must remain viable until they reach intestines. As a food component the added probiotic preparation should not affect neither the sensory properties of food product nor its texture.

Development of the technology leading to new food supplements containing microencapsulated probiotic bacteria and omega-3 fatty acids is a field of research where our group has been actively involved. As demonstrated here microencapsulation by spray-drying is an effective way to obtain high quality preparations of probiotic microorganisms combined with polyunsaturated omega-3 fatty acids. Initial studies showed that such a preparation can supplement many food products adding value to their nutritional benefits.

The research was founded by the project: „Health promoting food additives containing immobilized unsaturated fatty acids and pro biotic bacteria obtained by spray drying" co-financed by the European Union under Action 1.3.1, is co-financed by the European Regional Development Fund in the

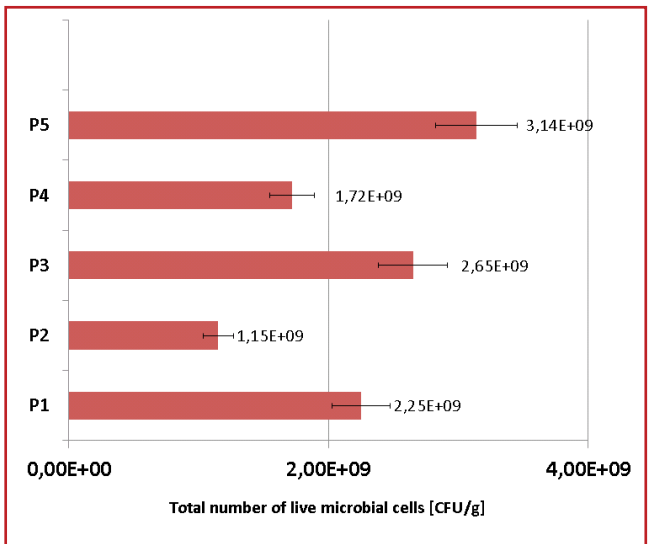

Fig. 4. The number of live cells of probiotic microorganisms in 5 spray-dried preparations (P1-P2 – *S.boulardii*, P3-P5 *L.plantarum*).

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framework of Innovative Economy Operational Programme 2007-2013.

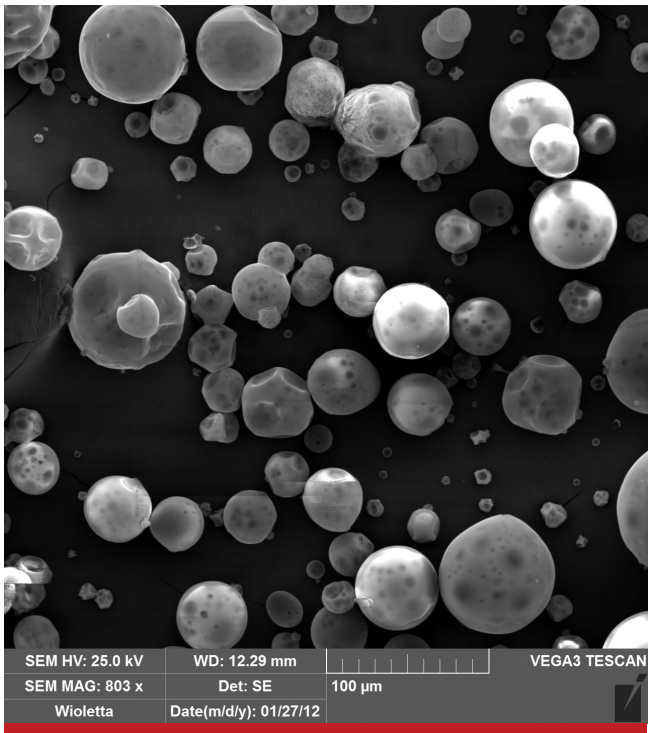

Fig. 5. Microcapsules obtained by spray-drying containing 2 strains of probiotic bacteria (LGG and Lp299v) and PUFA.

References

Chen, M.J., Chen, K.N., 2007. Applications of probiotic encapsulation in dairy products. In: Lakkis, Jamileh M. (Ed.), Encapsulation and Controlled Release Technologies in Food Systems. Wiley-Blackwell, USA, pp. 83–107

Kekkonen, R.A., Vasankari, T.J., Vuorimaa, T., Haahtela, T., Julkunen, I., Korpela, R.,2007. The effect of probiotics on respiratory infections and gastrointestinal symptoms during training in marathon runners. International Journal of Sport Nutrition and Exercise Metabolism 17 (4), 352–363.

Picot A and Lacroix C., (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yogurt. InternationalDairy Journal 14 505-515

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BIBLIOGRAPHY

Journal of Microencapsulation
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- ***Fate of nanostructured lipid carriers (NLCs) following the oral route: design, pharmacokinetics and biodistribution***
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  Journal of Microencapsulation Feb 2014, Vol. 31, No. 1: 1–8.
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  Chan Zhang, Yuefeng Dong, Liangqi Zhao
  Journal of Microencapsulation Feb 2014, Vol. 31, No. 1: 9–15.
- ***Polymer encapsulation of amoxicillin microparticles by SAS process***
  A. Montes, E. Baldauf, M. D. Gordillo, C. M. Pereyra, E. J. Martínez de la Ossa
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  Journal of Microencapsulation Feb 2014, Vol. 31, No. 1: 23–30.
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  Journal of Microencapsulation Feb 2014, Vol. 31, No. 1: 31–40.
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  Mirella Falconi, Stefano Focaroli, Gabriella Teti, Viviana Salvatore, Sandra Durante, Benedetta Nicolini, Isabella Orienti
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  A. Karewicz, K. Zasada, D. Bielska, T. E. L. Douglas, J. A. Jansen, S. C. G. Leeuwenburgh, M. Nowakowska
  Journal of Microencapsulation Feb 2014, Vol. 31, No. 1: 68–76.
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  Journal of Microencapsulation Feb 2014, Vol. 31, No. 1: 77–85.
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  Journal of Microencapsulation Feb 2014, Vol. 31, No. 1: 100–104.

Journal of Microencapsulation
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http://informahealthcare.com/toc/mnc/31/2

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BIBLIOGRAPHY

Journal of Microencapsulation Mar 2014, Vol. 31, No. 2: 147–155.

- ***Co-encapsulation of human serum albumin and superparamagnetic iron oxide in PLGA nanoparticles: Part II. Effect of process variables on protein model drug encapsulation efficiency***
  Quazi T. H. Shubhra, Tivadar Feczkó, Andrea F. Kardos, Judit Tóth, Hana Mackova, Daniel Horak, György Dósa, János Gyenis
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  Katarina Smilkov, Tanja Petreska Ivanovska, Lidija Petrushevska Tozi, Rumenka Petkovska, Jasmina Hadjieva, Emil Popovski, Trajce Stafilov, Anita Grozdanov, Kristina Mladenovska
  Journal of Microencapsulation Mar 2014, Vol. 31, No. 2: 166–175.
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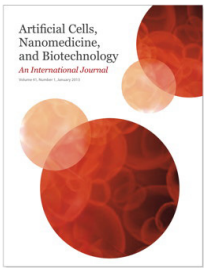

193–201.

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- ***Preparation of magnetically responsive albumin nanospheres and in vitro drug release studies***
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  Artificial Cells, Nanomedicine, and Biotechnology Feb 2014, Vol. 42, No. 1: 18–26.
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  Artificial Cells, Nanomedicine, and Biotechnology Feb 2014, Vol. 42, No. 1: 27–31.
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  Artificial Cells, Nanomedicine, and Biotechnology Feb 2014, Vol. 42, No. 1: 47–57.
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  Artificial Cells, Nanomedicine, and Biotechnology Feb 2014, Vol. 42, No. 1: 58–62.
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  Artificial Cells, Nanomedicine, and Biotechnology Feb 2014, Vol. 42, No. 1: 63–69.
- ***Stem cell suspension injected HEMA-lactate-dextran cryogels for regeneration of critical sized bone defects***
  Nimet Bölgen, Petek Korkusuz, İbrahim Vargel, Emine Kılıç, Elif Güzel, Tarık Çavuşoğlu, Duygu Uçkan, Erhan Pişkin
  Artificial Cells, Nanomedicine, and Biotechnology Feb 2014, Vol. 42, No. 1: 70–77.

Artificial Cells, Nanomedicine and Biotechnology

Vol. 42, Nb 2 (April 2014)

http://informahealthcare.com/toc/abb/42/2

- ***The properties research of polymerized human pla-***

BIBLIOGRAPHY

centa hemoglobin before and after lyophilization
Lanzhen Zhao, Wentao Zhou, Jinfeng Wang, Shen Li, Gang Chen, Jiaxin Liu, Chengmin Yang
Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 78–82.

- ***Isovolemic hemodilution with glutaraldehyde-polymerized human placenta hemoglobin (PolyPHb) attenuated rat liver ischemia/reperfusion injury***
  Zhen You, Qian Li, Bei Li, Chengmin Yang, Jin Liu, Tao Li
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 83–87.
- ***Radiolabelled nanohydroxyapatite with 99mTc: perspectives to nanoradiopharmaceuticals construction***
  Marta de Souza Albernaz, Carlos Alberto Ospina, Alexandre Malta Rossi, Ralph Santos-Oliveira
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 88–91.
- ***Hypothesis of peripheral nerve regeneration induced by terminal effectors***
  Bao Guo Jiang, Xiao Feng Yin, Pei Xun Zhang, Na Han, Yu Hui Kou
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 92–94.
- ***Oleic acid vesicles: a new approach for topical delivery of antifungal agent***
  Shivani Verma, Ankur Bhardwaj, Mohit Vij, Pawan Bajpai, Nishant Goutam, Lalit Kumar
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 95–101.
- ***Cell-matrix Interactions of Factor IX (FIX)-engineered human mesenchymal stromal cells encapsulated in RGD-alginate vs. Fibrinogen-alginate microcapsules***
  Bahareh Sayyar, Megan Dodd, Leah Marquez-Curtis, Anna Janowska-Wieczorek, Gonzalo Hortelano
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 102–109.
- ***BML-111, a lipoxin receptor agonist, ameliorates 'two-hit'-induced acute pancreatitis-associated lung injury in mice by the upregulation of heme oxygenase-1***
  Ying Zhen Wang, You Cheng Zhang, Jun Sheng Cheng, Qian Ni, Pei Jie Li, Shi Wen Wang, Wei Han, Yu Long Zhang
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 110–120.
- ***Lipase release through semi-interpenetrating polymer network hydrogels based on chitosan, acrylamide, and citraconic acid***
  Mehlika Pulat, Gülen Oytun Akalın, Nilay Demirkol Karahan
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 121–127.
- ***Molecularly imprinted cryogels for carbonic anhydrase purification from bovine erythrocyte***
  Murat Uygun, A. Alev Karagözler, Adil Denizli
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 128–137.
- ***Bitargeting and ambushing nanotheranostics***
  Rıdvan Say, Lokman Uzun, Suzan Yazar, Adil Denizli, Deniz Hür, Filiz Yılmaz, Arzu Ersöz
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 138–145.
- ***The effect of pretreatment with substrates on the activity of immobilized pancreatic lipase***
  Guliz Ak, Yeser Aktuna, Funda Kartal, Ali Kilinc
  Artificial Cells, Nanomedicine, and Biotechnology Apr 2014, Vol. 42, No. 2: 146–150.

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ARTICLE

Encapsulated probiotic bacteria in supplements and foods

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Scope

Probiotic bacteria are added to many foods and nutraceutical products. It is a challenge to keep these beneficial cultures alive during processing and storage. Microencapsulation has been shown to improve their stability in these situations, and a few products which have been marketed will be presented. However, foods and supplements having microencapsulated cultures only comprise a small fraction of the market. This manuscript will describe the microencapsulation techniques used. Some reasons why it is not as extensively used as expected will be presented, as well as some opportunities for innovation.

Foods

A previous report mentioned that cereals, yoghurt and chocolate were the first food products having ME cultures (Champagne and Kailasapathy, 2011). Since YogActive (Figure 1) three similar cereals with probiotic "pearls" were launched but they were not commercial successes.

Chocolate bars with ME cultures are still found, but remain specialty products. This matrix has indeed been shown to be a good carrier for extended storage of the cultures (Possemiers et al.,2010). What have appeared recently are chocolate bites (Figure 2). The particularity of this product is that it contains a billion cells per bite, which only contain 10 calories. Such a small quantity can also classify the product as a supplement. The producer guarantees a 1 billion CFU/ portion level after 2 years of storage a room temperature. Presumably, microencapsulation is instrumental in allowing this achievement, but the company still has to add 10 billion cells at manufacturing to allow for viability losses during storage. Furthermore, it uses a probiotic species which is much more stable during storage than those from the genera *Lactobacillus* and *Bifidobacterium*: *Bacillus coagulans*. Therefore, success is linked to a combination of 3 elements .

The dairy sector is the one which has the highest number of products having probiotic cultures but, to my knowledge, none are microencapsulated (ME). A yoghurt containing ME bifidobacteria was launched in Mexico but was discontinued. Presumably this is because, in yoghurt, cheese or fluid milk, the dairy industry prefers that the addition of probiotics does not modify the flavour or the texture of foods.

Why so few foods?

First of all, ME cultures cost approximately twice as much than the free-cell cultures do. Therefore, the ME cultures must reduce the viability losses by a factor of at least two in order to become economically viable.

The second problem is size. Frozen or died products of free cells release the bacteria into the matrix and do not modify texture our mouth feel. The major commercial ME cultures available have particle sizes greater than 100 µm, which will affect the sensory properties of foods. Unfortunately, many microencapsulation processes increase particle size. For example, spray-coating of a freeze-dried culture increases the average size of the particles by approximately 40% (Champagne et al. 2010). A greater number of applications

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would only seem possible if consumers accept the visible presence of ME cultures such as in the cereal product (Figure 1). This actually is an opportunity for innovation. Like cereals, many food products do not have a uniformly smooth texture and could accommodate probiotic particles. Examples include: cereal bars, fermented vegetables, tofu, salads, tempeh.

Another problem could be the desire to obtain health claims. There is a wealth of data that show that microencapsulation improves the survival of probiotics to the gastric environment. Thus, in cases where cell viability in the gastro-intestinal tract is considered essential for bioactivity, ME cultures would theoretically be much better than free-cell ones. But one can imagine that regulatory authorities will demand clinical proof. If a wealth of clinical data has been obtained with free-cell cultures, there is limited desire to repeat them with ME cultures. However, companies which are preparing new clinical trials should certainly consider incorporating a ME culture in the experimental plan.

Supplements

A more extensive use of ME for probiotics has been in the food supplements sector. In this market, powders are traditionally ME and placed into caplets (Figure 1) or pills. In the early products the most widely used technology was spray-coating.

Figure 1: Examples of initial formats of supplements and food products containing microencapsulated probiotics. From: www.webbernaturals.com and www.yogactive.com.

Newer technologies based on alginate particles have now appeared. Micropharma Ltd has an alginate/poly-L-lysine/

alginate system (Martoni et al, 2001) applied to its Cardioviva™ *Lactobacillus reuteri* culture. Newer entries are the Intellicaps® (Brace/VesalePharma) and ProGel (Uniquest) systems. Other available techniques for industry are based on spray-drying (MicroMAX; CSIRO) or compression (BioTract – Nutraceutix; Biovelia). Unfortunately, no commercial food or supplement products presently claim the use of these technologies.

But there are new products on the market (Figure 2). They are lipid based. As in the cereal (Figure 1) the new trend in supplements is large "pearls" (Figure 2). Presumably they are obtained by extrusion of a molten lipid carrier, containing a probiotic culture powder, on a cold surface or in a cold liquid. Spray chilling or spray-congealing technologies could also be involved. As was initiated by Micropharma, the trend is towards multiple protection encapsulation or protection systems. In the case of the ProBiotic bites, a coating of dark chocolate provides a protective layer, which the supplier claims has antioxidants.

Figure 2: Examples of « pearls » or « bites ».
From: www.natureswaycanada.ca and www.probioticbites.com.

Future opportunities?

Since companies are under a legal obligation to obtain the CFU levels claimed on the label at the date of expiration, the primary interest in microencapsulation is to enhance survival of the probiotics to processing and storage. But it is foreseeable that viability benefits during consumption and in the GI tract will eventually also be considered.

There are increasingly dry products which are rehydrated immediately prior to consumption (Figure 3).Our experience with the inoculation of freeze-dried cultures in foods is to the effect that rehydration conditions are critical to viability. This is also well documented for bacterial analyses (Muller et al. 2010). The temperature of hydration should ideally be between 30-37°C and the pH of the medium is preferably between 6 and 7. With respect to infant nutrition, there is the possibility that the meal would be rehydrated with water too hot or too cold. With the athlete high-protein powder it is conceivable that the product could be added to a fruit juice. In both cases extensive viability losses would occur. Microencapsulation could be used to actually prevent rehydration under these circumstances and only enable cell release in the duodenum. The efficacy of the products would not be subject to potentially detrimental consumer practices.

There is therefore cause for optimism for the development of foods with ME probiotics. In addition to the traditional benefits during processing and storage, it is to be hoped that industry and consumers will also take advantage of innovative formats, as well as improved viability during rehydration and in the GI tract of ME cultures.

Figure 3: Examples of products containing probiotic bacteria which must be rehydrated before consumption.
From: www.nestle-baby.ca ; www.dpsnutrition.ne

References

Champagne C.P., Raymond Y., Tompkins T.A. 2010. The determination of viable counts in probiotic cultures microencapsulated by spray coating. Food Microbiology. 27: 1104-1111.

Champagne C.P., Kailasapathy K. 2011. Some current food products with microencapsulated probiotics. Agro FOOD Industry Hi Tech, 22 (2): 54-56.

Martoni, C., Jones, M.L., Prakash, S., 2011. Epsilon Poly-L-Lysine capsules. Patent WO/2011/075848 (PCT/CA2010/002057

Muller, J.A., Stanton, C., Sybesma, W., Fitzgerald, G.H., Ross, R.P., 2010. Reconstitution conditions for dried probiotic powders represent a critical step in determining cell viability. Journal of Applied Microbiology 108, 1369-1379.

Possemiers S., Marzorati M., Verstraete W., Van de Wiele, T., 2010. Bacteria and chocolate: A successful combination for probiotic delivery. International Journal of Food Microbiology 141: 97–103.

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ARTICLE

Cellular bioencapsulation: Looking back on the future

Hani Al-Salami, and TK Mukkur, Curtin University, Australia

Cellular microencapsulation is the most promising field of research with endless health benefits.

The idea of bacterial and mammalian cell microencapsulation has been explored for many decades, with the aim being to optimise viability, maintain physiological functionality and provide a matrix for targeted delivery in a safe and robust manner.

Mammalian Cell Bioencapsulation

Since the innovation of Artificial-Cell Microencapsulation (ACM) technology by Thomas Chang at McGill University (Canada) in the 1960s, this technology has been used worldwide by many researchers, scientists and translational entrepreneurs. Artificial cells are engineered particles that mimic the biological cells and are novel delivery systems for biologically active molecules. ACM has been used significantly in the delivery of various cells and therapeutics [Chang, 1999 ; Chang, 1998]. In ACM, the polymeric semi-permeable membrane encapsulates biotherapeutics (e.g. cells) and biologically active compounds (e.g. bile acids), and allows the diffusion of small molecules such as nutrients, oxygen and biowastes, while preventing metabolising enzymes, antibodies, cytokines, and complements factors from entering the microcapsule. Interestingly, ACM membrane can provide protection for the delivery of viable bacterial and mammalian cells during handling and also from the immune system after administration. Another main advantage from using ACM is that it provides a targeted delivery of the molecules to the site of action in the gut "pH controlled delivery", such as the colon [Negrulj, 2013 ; Martinez, 2014]. Various factors need to be considered when selecting a polymer to form a membrane to encapsulate a therapeutic agent/mixture. Firstly, the extent of polymer's biocompatibility with the encapsulated biotherapeutics. Secondly, the polymeric membrane should be permeable for nutrients, oxygen and waste, to support cell proliferation, metabolism and differentiation, but impermeable for immune system. Thirdly, the membrane should have enough mechanical strength to deliver the full dose of the cells including cellular vaccines or the bioactive compound including antigens, and other bioactive molecules to the site of action in the GIT. A common excipient used in cellular microencapsulation is alginate poly-L-lysine.

With the continuous interests in ACM applications, many labs are aiming to produce more complex, sophisticated and multilayered systems that are capable of maintaining viable cells, bacteria or mammalian cells that can perform normal physiological tasks in the body. Such aim can have great future implications as the fields of probiotics and stem cell continue to grow including undifferentiated and differentiated mammalian cells (pluripotent and multipotent stem cells). This is particularly interesting as this technology offers many advantages, compared with other formulation methods. Such advantages include formulation process that is gentle and does not need heating or significant pressure and the use of biodegradable and biocompatible polymers, which can be given internally with minimum complications. In addition to aiming for least immune-response associated with stem cell implantation and vaccine delivery is another area of great interest. Live-attenuated bacteria, which triggers both arms of the immune system is another area that can utilise the ever more complex microencapsulation systems for best results while maintaining safety [4]. Characterising of such microcapsules can be easily performed. A good example is our 'platform' microcapsules developed in our laboratory. Figure 1 shows this system using Light Microscope (Figure 1–a), Scanning Electron Microscope (Figure 1-b) and Energy-Dispersive X-ray Spectroscopy (Figure 1-c).

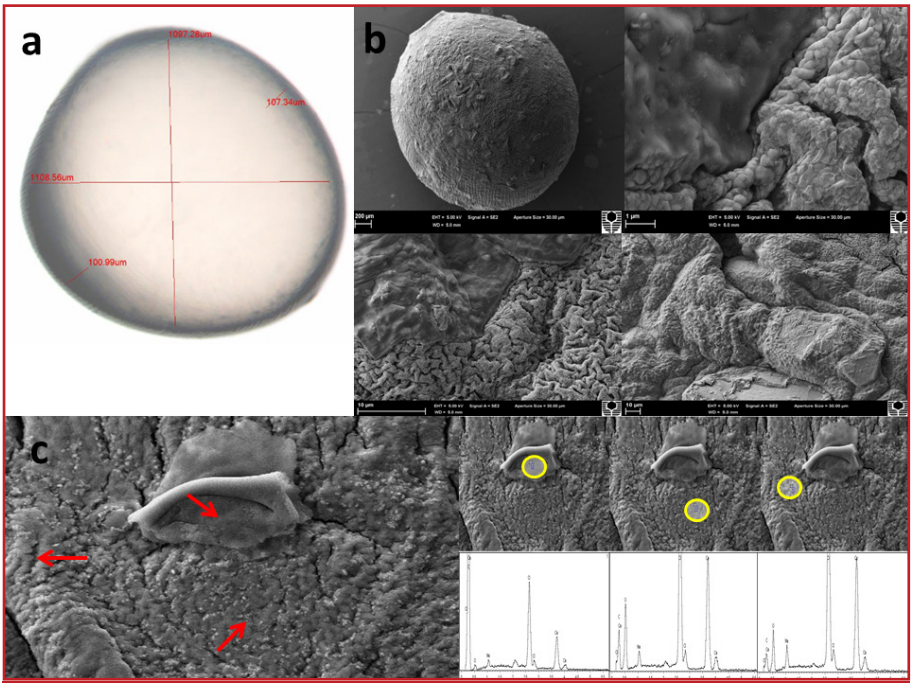

Figure 1: Complex multicompartmental microcapsules, (a) Light microscopy image (b) Scanning Electron Micrographs at various scales, (c) Energy-Dispersive X-ray spectroscopy with 3 points analysis.

ACM technology is expected to progress rapidly in the near future with its versatility placing it as a major destiny for many applications, especially in the area of undifferentiated and differentiated mammalian cells, as well as vaccine delivery. Thus, future microcapsules are anticipated to incorporate multicompartments, multilayers and multitherapetuic systems that will out-deliver current microcapsules (Figure 2).

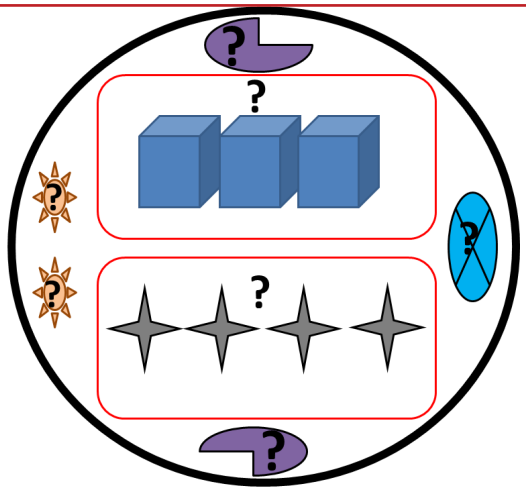

Figure 2: Future microcapsules: many unknown molecules incorporated into a single system.

Vaccine Bioencapsulation

Generation of a relevant type of antibody and cell-mediated immune response against the critical virulence antigens of pathogens is crucial in the development of vaccines capable of providing long-term protection against the disease syndromes. Another that impacts on the compliance with the recommended vaccination schedules is the affordability of the vaccines by socially disadvantages populations. The currently marketed vaccines used in infants and adolescents are highly priced, hence the need to develop vaccines that are easy to manufacture cost-wise resulting in cheaper vaccines becoming available to the public. This is exactly the reason that we have expended our in developing vaccines, for example, against whooping cough that will cost a lot less than the currently used vaccines against infectious diseases. One way to reduce the cost of vaccines is to develop DNA-based vaccines. However, there are three alternatives that may provide better vaccination agenda.

One possibility is to search for adjuvants that are non-toxic and will predictably induce both arms of the specific immune responses required for long-term protection against many infectious diseases. One adjuvant approved by regulatory authorities worldwide is Alhydrogel, with a different modified adjuvant containing monophosphoryl Lipid A [agonist of Toll-Like Receptor 4] and aluminium hydroxide [AS04] used with the HPV vaccine. Another adjuvant is a sequalene-based adjuvant called MF59®, a squalene-based adjuvant. This has been used with the swine flu vaccine [Fluad] which is approved for use in people who are 65 years old or older by the FDA. However, search for a universal adjuvant capable of inducing both arms of the immune response is ongoing.

While microencapsulation has been used with model inert antigens for the most part, our research team include the leader of the Infectious Disease Vaccine Development Group at Curtin University, Associate Professor TK Mukkur. Our group has a special interest in exploring the potential of microencapsulation technology in delivery of vaccine antigens associated with the induction of both arms of the immune response, with or without co-administration of probiotics, as novel delivery systems. The rationale underpinning this approach is to be able to generate tailored immune responses capable of providing long term protection eliminating the need for cold chain, particularly in developing nations, resulting in better health outcomes for humans as well as food-producing animals.

References

Chang, T.M., Microencapsulation of enzymes, cells, and genetically engineered microorganisms. Methods Mol Med, 1999. 18: p. 315-30.

Chang, T.M., Pharmaceutical and therapeutic applications of artificial cells including microencapsulation. Eur J Pharm Biopharm, 1998. 45(1): p. 3-8.

R. Negrulj, A. Mooranian, and H. Al-Salami*, Potentials and Limitations of Bile Acids in Type 2 Diabetes Mellitus: Applications of Microencapsulation as a Novel Oral Delivery System. Journal of Endocrinology and Diabetes Mellitus, 2013. 1(2): p. 49-59.

Armin Mooranian, Jorge Martinez, Rebecca Negrulj, Sangeetha Mathavan, Jessica Sciarretta, Nigel Chen-Tan, TK Mukkur, Momir Mikov, Mladena Lalic-Popovic, Maja Stojančević, Svetlana Golocorbin-Kon, Hani Al-Salami*. A complex microencapsulated system: a platform for optimised oral delivery of antidiabetic drug-bile acid formulations. Pharmaceutical Development and Technology; 2014 (in press)

Hani Al-Salami

Dr Hani Al-Salami is a registered Australian and New Zealand health professional with expertise in the area of bioencapsulation, and drug delivery in diabetes. His research covers microencapsulation technology, and the applications of bile acids and probiotics in diabetes. Dr Al-Salami's research at Otago School of Pharmacy (New Zealand), McGill Faculty of Medicine (Canada) and now at Curtin School of Pharmacy (Australia) have produced and continue to produce significant publications, industrial links and translational findings.

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Looking for a PhD position
Trần Hải Đăng

Lecturer of Institute of Biotechnology and Environment, Nha Trang University
Organizer of VIth training school on Bioencapsulation in Nha Trang, Vietnam, 2014
Master on Science, Technology and Health, mention on Food Science at AgroSup Dijon, Burgundy University, France, I'm now working as lecturer and researcher in Nha Trang University, Vietnam. My research domain focuses on encapsulation of natural compounds for use in food and feed purpose. I'm actually principal investigator of a national project on microencapsulation of Vietnamese essential oil (Gac oil, ginger oil, garlic oil). I have experience on working with polymers as gelatin, chitosan, alginate, carrageenan and maltodextrin used as coating materials in coacervation, dripping, gelation or spray-drying methods. Hoping go farther and have deeply knowledge in this domain, I'm looking for a PhD position which focuses on microencapsulation of bioactive compounds for application in food and environment.

Contact: **Trần Hải Đăng**
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PhD position in microfluidics

The research fields of the Nanostructured Assemblies team at the Biopolymers Interactions Assemblies Research Unit in Nantes (France) include elaboration of functional assemblies/materials that take advantage of the information provided by biopolymers. The team's activities are divided into analogs and biomimetic assemblies model of plant cell wall and bio-sourced innovative nano(micro)structured materials.
Nanostructured Assemblies team has an open position in the field of constrained biopolymers assembly using droplets microfluidics. The research concerns the conception of microparticles with innovative architecture based on mixtures of biopolymers. Plant protein-polysaccharide mixtures will be considered in this project. Water-in-oil and water-in-water emulsion using droplets microfluidics will be used to generate "green microparticles" for new food ingredients and controlled release or cell biology applications. This fundamental research activity will be conducted during three years in the frame of the Institut National de la Recherche Agronomique (INRA) - Region Pays de Loire.

Application deadline: 30/04/2014

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More info & contact: http://we.experiencepg.com (reference RND00002660); Susana Fernández Prieto, fernandezprieto.s@pg.com.

Milk functional ingredients effect on probiotic encapsulation

Université de Lorraine (FRANCE) - Laboratoire d'Ingénierie des Biomolécules

This ministerial thesis scholarship will be focused on the use of whey proteins in order to enhance the probiotic efficiency of Lactobacillus rhamnosus GG (LGG). To improve the encapsulation efficiency, interactions between LGG and milk proteins will be investigated at three levels: molecular, micro and macro-scales. This PhD subject will ensure the continuity with the work of a previous student (J. Burgain, 2013). Indeed, a successful encapsulation was found to be strongly linked to the presence of various cell surface features consisting of proteins (pili, polysaccharides...). In order to go further and to identify specifically which biomolecules at the surface of LGG interact with the whey proteins, some mutants derived from the LGG wild-type strain will be used. Strong competences in food engineering and physico-chemistry will be requested to the candidates.

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Bioencapsulation Research Group is a non-profit association promoting networking and research in the encapsulation technology of bioactives. It organises academic conferences and industrial symposiums, publishes newsletters and manages a website.

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